PRIMARY EYE IRRITATION STUDY IN RABBITS

TEST METHOD NO.:	P202
STUDY NUMBER:	8821
SPONSOR:	SYNTROLEUM CORPORATION 1350 South Boulder, Suite 1100 Tulsa, OK 74119-3295
TEST SUBSTANCE IDENTIFICATION:	RDIL 0486
TEST SUBSTANCE DESCRIPTION:	Clear colorless liquid
DATE RECEIVED:	February 18, 2000
PSL REFERENCE NO.:	E00218-1D
DATES OF TEST:	March 1-4, 2000
NOTEBOOK NO.:	00-05; pages 312-321

1. PURPOSE

To provide information on the irritation likely to arise from a single instillation of RDIL 0486 into the eye.

2. PROCEDURE

A group of New Zealand albino rabbits was received from Davidson's Mill Farm, South Brunswick, NJ. The animals were singly housed in suspended stainless steel caging with mesh floors. Litter paper was placed beneath the cages and was changed at least three times per week. The animal room was temperature controlled and had a 12-hour light/dark cycle. The animals were fed Purina Rabbit Chow #5326 and filtered tap water was supplied *ad libitum* by an automatic watering system.

Following acclimation to the laboratory, the eyes of a group of animals were examined. Six healthy rabbits (3 male and 3 female) without pre-existing ocular irritation were selected for test. One-tenth of a milliliter of the test substance was instilled into the conjunctival sac of the left eye of each rabbit by pulling the lower lid away from the eyeball. The upper and lower lids were then gently held together for about one second before releasing to minimize loss of the test substance. The right eye remained untreated and served as a control.

Ocular irritation was evaluated with the illumination of a white light source at 1, 24, 48 and 72 hours after instillation according to the "Scale for Scoring Ocular Lesions"[1] (See Table 2). Fluorescein dye

[1] Draize, J.H., Woodward, G. and Calvery, H.O. Methods for the study of irritation and toxicity of substances applied topically to the skin and mucous membranes. *J. Pharmacol. Exp. Ther.* 1944; 82:377-390.

was used at 24 hours to verify the absence of corneal damage. The time interval with the highest mean score (Maximum Mean Total Score - MMTS) for all rabbits was used to classify the test substance by using the system of Kay and Calandra[1] described below.

MMTS	Irritation Classification	Requirement For Maintenance of Classification[1]
0.0 - 0.5	Non	Up to 0.5 at 1 hour with zeros at 24 hours; otherwise, increase one level
0.6 - 2.5	Practically non	with zeros at 24 hours; otherwise, increase one level
2.6 - 15.0	Minimally	with zeros at 48 hours; otherwise, increase one level
15.1 - 25.0	Mildly	with zeros at 96 hours; otherwise, increase one level
25.1 - 50.0	Moderately	with 7 day mean $\leq$ 20 and individual total scores $\leq$ 10 in at least 60% of the rabbits with no total score >30; otherwise, increase one level
50.1 - 80.0	Severely	with 7 day mean $\leq$ 40 and individual total scores $\leq$ 30 in at least 60% of the rabbits with no total score > 60; otherwise, increase one level
80.1 - 100.0	Extremely	with 7 day mean $\leq$ 80 and individual total scores $\leq$ 60 in at least 60% of the rabbits with no total score >100; otherwise, increase one level
100.1 - 110	Maximally	with 7 day mean > 80 and individual total scores > 60 in at least 60% of the rabbits; otherwise, decrease one level

3. RESULTS

Individual eye irritation scores are presented in Table 1. The Draize Scale for Scoring Ocular Lesions is presented in Table 2.

All animals appeared active and healthy. Apart from the eye irritation noted below, there were no other signs of gross toxicity, adverse pharmacologic effects or abnormal behavior.

One and twenty-four hours after test substance instillation, conjunctivitis was observed in all six treated eyes. Within 48 hours, all rabbits were free from ocular irritation.

The Maximum Mean Total Score of RDIL 0486 is 3.7

4. CONCLUSION

Based on the classification system used, RDIL 0486 is classified as minimally irritating to the eye.

[1] Kay JH, and Calandra JC. Interpretation of eye irritation tests. *J Soc Cos Chem* 1962; 13:281-289.

SIGNATURES

RDIL 0486

We the undersigned declare that the methods, results and data contained in this report faithfully reflect the procedures used and raw data collected during the study.

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Date

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TABLE 1: INDIVIDUAL SCORES FOR OCULAR IRRITATION

	Rabbit No.: 1006 (Male)				Rabbit No.: 1007 (Female)				Rabbit No.: 1008 (Male)			
	Hours				Hours				Hours			
	1	24	48	72	1	24	48	72	1	24	48	72
I. Cornea												
A. Opacity	0	0[1]	0	0	0	0[1]	0	0	0	0[1]	0	0
B. Area	4	4	4	4	4	4	4	4	4	4	4	4
(AxB)x5	0	0	0	0	0	0	0	0	0	0	0	0
II. Iris												
A. Values	0	0	0	0	0	0	0	0	0	0	0	0
Ax5	0	0	0	0	0	0	0	0	0	0	0	0
III. Conjunctivae												
A. Redness	1	1	0	0	2	1	0	0	2	1	0	0
B. Chemosis	0	0	0	0	0	0	0	0	0	0	0	0
C. Discharge	0	0	0	0	1	0	0	0	0	0	0	0
(A+B+C)x2	2	2	0	0	6	2	0	0	4	2	0	0
Total	2	2	0	0	6	2	0	0	4	2	0	0

[1] 2% fluorescein sodium used to verify the absence of corneal opacity.

TABLE 1 (cont.): INDIVIDUAL SCORES FOR OCULAR IRRITATION

	Rabbit No.: 1009 (Female)				Rabbit No.: 1010 (Male)				Rabbit No.: 1011 (Female)			
	Hours				Hours				Hours			
	1	24	48	72	1	24	48	72	1	24	48	72
I. Cornea												
A. Opacity	0	0[1]	0	0	0	0[1]	0	0	0	0[1]	0	0
B. Area	4	4	4	4	4	4	4	4	4	4	4	4
(AxB)x5	0	0	0	0	0	0	0	0	0	0	0	0
II. Iris												
A. Values	0	0	0	0	0	0	0	0	0	0	0	0
Ax5	0	0	0	0	0	0	0	0	0	0	0	0
III. Conjunctivae												
A. Redness	1	1	0	0	2	1	0	0	2	1	0	0
B. Chemosis	0	0	0	0	0	0	0	0	0	0	0	0
C. Discharge	0	0	0	0	0	0	0	0	0	0	0	0
(A+B+C)x2	2	2	0	0	4	2	0	0	4	2	0	0
Total	2	2	0	0	4	2	0	0	4	2	0	0

[1] 2% fluorescein sodium used to verify the absence of corneal opacity.

TABLE 2: SCALE FOR SCORING OCULAR LESIONS

1. Cornea

A. Opacity-degree of density (area most dense taken for reading)

No Opacity 0
Scattered or diffuse area, details of iris clearly visible 1*
Easily discernible translucent areas, details of iris slightly obscured 2*
Opalescent areas, no details of iris visible, size of pupil barely discernible 3*
Opaque, iris invisible 4*

B. Area of cornea involved

One quarter (or less) but not zero 1
Greater than one quarter, but less than half 2
Greater than half, but less than three quarters 3
Greater than three quarters, up to whole area 4

A X B X 5 Total Maximum = 80

2. Iris

A. Values

Normal 0
Folds above normal, congestion, swelling, circumcorneal injection (any or all of these or combination of any thereof) iris still reacting to light (sluggish reaction is positive) 1*
No reaction to light, hemorrhage, gross destruction (any or all of these) 2*

A X 5 Total Maximum = 10

3. Conjunctivae

A. Redness (refers to palpebral and bulbar conjunctivae excluding cornea and iris)

Vessels normal 0
Vessels definitely injected above normal 1
More diffuse, deeper crimson red, individual vessels not easily discernible 2*
Diffuse beefy red 3*

B. Chemosis

No swelling 0
Any swelling above normal (includes nictitating membrane) 1
Obvious swelling with partial eversion of lids 2*
Swelling with lids about half-closed 3*
Swelling with lids about half-closed to completely closed 4*

C. Discharge

No discharge 0
Any amount different from normal (does not include small amounts observed in inner canthus of normal animals) 1
Discharge with moistening of the lids and hairs just adjacent to lids 2
Discharge with moistening of the lids and hairs, and considerable area around the eye 3

Score (A + B + C) X 2 Total Maximum = 20

Total Maximum Score: 110 represents the sum of all scores obtained for the cornea, iris and conjunctivae.

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* These scores represent a positive response.